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# Selection of High-Affinity Synergistic Antibodies from an scFv-Phage Library

## Overview

The binding of a monoclonal antibody to a protein antigen sometimes induces a conformational change in the antigen, which facilitates the subsequent binding of a second monoclonal antibody at a distinct epitope. Such synergistic antibody pairs bind to their antigen with a higher affinity than do the individual antibodies binding independently to the antigen. To date, these antibody pairs have been identified by surveying collections of existing monoclonal antibodies. The goal of this project is to develop a directed approach for isolating these synergistic antibodies.

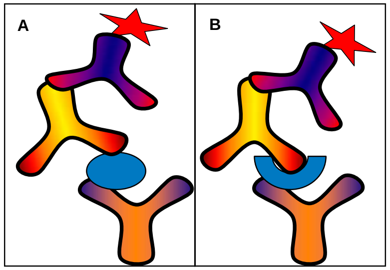
Sandwich immunoassays (ELISAs) are used to detect a wide variety of molecules. The sensitivity of the assay is largely dependent on the affinity of the monoclonal detection antibodies used in the sandwich. By increasing the affinity of these antibodies, we can significantly increase the sensitivity (and the specificity) of the assay for the target antigen.

Directed selection of synergistic antibodies from an scFv-phage library would allow for isolation of antibody combinations with higher affinity for the antigen than could ordinarily be selected. Careful design of the strategy used for screening the scFv-phage library would allow the researcher to identify those antibodies with binding characteristics that can be exploited for a variety of specific applications.

## Hypothesis and Research Approach

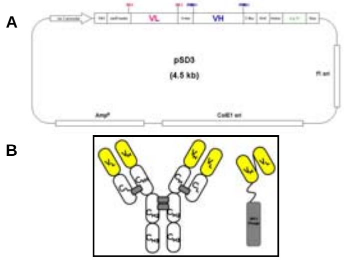
*Synergistic antibodies are an inherent part of the polyclonal antibody population produced during the immune response to an antigen and they can be selected from this population.*

We will use phage display technology to produce an scFv-phage immune library to Immunoglobulin A (IgA). IgA was chosen for these studies because of its protein secondary structure (primarily beta-pleated sheet) and because it may have value as a marker for fecal contamination. The immune library will be screened for clones that express antibodies that bind to IgA, as well as combinations of antibodies that bind with synergy to human IgA.



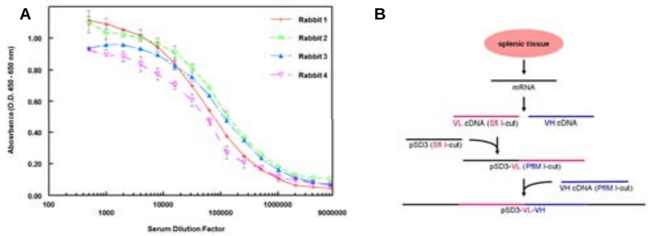

***Panel A) Sandwich ELISA.*** The target antigen is captured by a primary antibody. A second primary antibody is added and binds to the target antigen at another epitope. The complex is detected by incubation with an enzyme-conjugated secondary antibody, followed by a colorimetric substrate.

***Panel B) Sandwich ELISA with Synergistic Antibodies.*** Binding of the antigen by the first primary antibody facilitates a conformational change in the antigen which allows the second primary antibody to bind with greater affinity than is seen with a non-synergistic pair of antibodies.



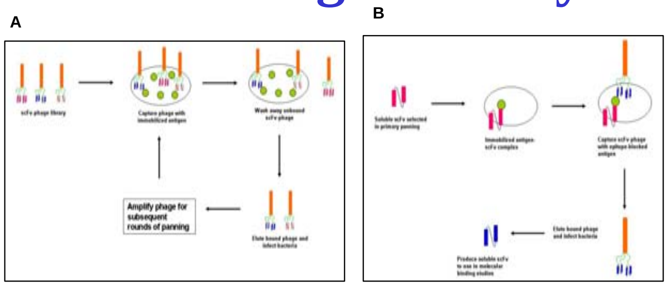

***Panel A)* pSD3 phagemid vector.** The phagemid vector used for the construction of the library contains unique restriction sites for insertion of the two scFv variable regions, as well as two epitope tags for scFv detection. The vector contains an Amber stop codon, which allows for soluble scFv expression, when desired.

***Panel B)* scFv structure.** The final scFv product is made up of the two antibody variable regions, linked together with a flexible peptide region.




***Panel A)* Rabbit immune response to IgA.** Serum from each immunized rabbit was serially diluted onto wells coated with 5 µg/mL of human IgA. Serum antibody binding was detected by a peroxidase-conjugated goat anti-rabbit IgG and reaction color was developed with TMB colorimetric substrate..

***Panel B)* Library cloning strategy.** Following harvest of the splenic RNA, cDNA encoding the light and heavy chain antibody variable regions was synthesized and digested with the appropriate restriction enzyme. The VL cDNA was inserted into the vector first, followed by the VH cDNA. The final genomic product is a cDNA library of different light and heavy chains in the pSD3 phagemid vector.




***Panel A)* Primary Panning Strategy.** scFv-phage will be selected based on their ability to bind to the antigen, IgA. Positive binders will be used to re-infect bacteria, in order to enrich for those clones that express scFv which bind to IgA. This panning scheme will be repeated 2-5 times to eliminate clones that bind non-specifically.

***Panel B)* Epitope-blocked selection strategy.** This selection strategy will be used to identify scFv that bind to distinct epitopes on the antigen. By blocking the initial epitope with soluble scFv, we are forcing the selection of scFv that bind to another epitope, thereby increasing the chance that selected scFv are capable of binding to IgA with synergy.

## Conclusions

An scFv-phage library to human IgA was constructed in 2005, but the library stocks were lost in the aftermath of hurricane Katrina. At this time, we are in the process of re-constructing the library, with procedural modifications designed to increase the affinity and diversity of the antibodies produced.

Future directions will focus on the identification of synergistic antibodies. We will employ library screening strategies to select for clones that express scFv that bind to IgA, as well as pairs of scFv that bind to distinct epitopes on IgA. Following identification of scFvs that bind to the antigen, molecular characterization of the binding interactions will be performed.

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